Hypothesis

Prion protein structural features indicate possible relations to signal peptidases

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Abstract Transmissible spongiform encephalopathies (TSEs) in mammalian species are believed to be caused by an oligomeric isoform, PrP^{Sc}, of the cellular prion protein, PrP^{C}. One of the key questions in TSE research is how the observed accumulation of PrP^{Sc}, or possibly the concomitant depletion of PrP^{C} can cause fatal brain damage. Elucidation of the so far unknown function of PrP^{C} is therefore of crucial importance. PrP^{C} is a membrane-anchored cell surface protein that possesses a so far unique three-dimensional structure. While the N-terminal segment 23–120 of PrP^{C} is flexibly disordered, its C-terminal residues 121–231 form a globular domain with three α-helices and a two-stranded β-sheet. Here we report the observation of structural similarities between the domain of PrP(121–231) and the soluble domains of membrane-anchored signal peptidases. At the level of the primary structure we find 23% identity and 41% similarity between residues 121–217 of the C-terminal domain of murine PrP and a catalytic domain of the rat signal peptidase. The invariant PrP residues Tyr-128 and His-177 align with the two presumed active-site residues of signal peptidases and are in close spatial proximity in the three-dimensional structure of PrP(121–231).

Key words: Transmissible spongiform encephalopathy; Cellular prion protein; Signal peptidase; Three-dimensional structure; Sequence similarity

1. Introduction

Prions are the infectious agents of fatal transmissible spongiform encephalopathies (TSEs) such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD) in humans (for reviews see [1–4]). The 'protein only' hypothesis [5–7] proposes that the pathogenic component in prions is an oligomeric, protease-resistant form, PrP^{Sc}, of the monomeric cellular prion protein, PrP^{C}. PrP^{Sc} is most likely identical with PrP^{C} in its covalent structure [8] but possesses a different tertiary structure [9,10] and may propagate by imposing its fold on PrP^{C} [11]. Research on the prion protein has long been dominated by studies of PrP^{Sc} because it can be purified in reasonable quantities from diseased brains, and its presence is related with the appearance of clinical symptoms of prion diseases. The discovery that PrP^{C} is a benign, host-encoded protein is of more recent origin [12]. Besides numerous uncertainties about the nature of the infectious agent [13] and the mechanism of propagation of PrP^{Sc} [4], one of the key questions in TSE research is how the observed accumulation of PrP^{Sc}, or possibly concomitant depletion of PrP^{C} during TSEs can cause neuronal cell death and fatal brain damage [1]. Elucidation of the natural function of PrP^{C} is therefore a most important goal. Although the presence of PrP^{C} is required for the development of TSEs [14,15], mice devoid of PrP^{C} develop normally [14], and only small abnormalities such as reduced survival of Purkinje neurons [16], altered sleep patterns [17] and possibly impaired synaptic function [18,19] have been reported for some strains of knock-out mice. Despite the nearly normal phenotypes of PrP-deficient mice, it cannot be excluded that PrP^{C} might have important functions in wild-type mice, since knock-out mice could possibly adapt during early development to the lack of PrP [1]. The high degree of sequence identity (generally above 90%) among the known mammalian prion protein sequences [20,21] could most readily be rationalized by the assumption of a life-supporting PrP^{C} function.

PrP^{C} from mammalians is a cell surface glycoprotein of 209 amino acids (residues 23–231; amino acid numbering according to human PrP [20]), which is anchored to the cell membrane via a glycosyl phosphatidyl inositol (GPI) anchor at its C-terminus and expressed in most cell types [2,8,20]. Rapid progress has recently been made in the physico-chemical and structural characterization of recombinant PrP^{C} expressed in *Escherichia coli*. Most importantly, the three-dimensional structure of recombinant PrP^{C} in solution has been determined [22–25]. While its N-terminal segment 23–120 is unstructured, the C-terminal segment PrP(121–231) forms a self-folding domain with three α-helices and a two-stranded β-sheet [22,26]. Due to the limited availability and the poor solubility of PrP^{Sc} it seems unlikely that correspondingly detailed structural and biochemical data will be obtained for this aggregated form of the prion protein in the near future. This situation further underlines the importance of continued investigations on PrP^{C}, since these may also lead to novel insights into the mechanisms which lead to the transformation of PrP^{C} into PrP^{Sc}.

Apart from the recent finding that the flexible N-terminal segment of PrP^{C} appears to bind Cu^{2+} ions in vitro [27] and in vivo [28], the function of PrP^{C} in the cell is still a mystery. This note describes new observations on the level of the primary and tertiary structure of PrP^{C} that indicate a possible relation to membrane-anchored signal peptidases. The initial motivation for this study came from the observation that

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preparations of recombinant mammalian prion proteins tend to be subject to partial proteolysis after prolonged incubation under the conditions used for nuclear magnetic resonance (NMR) spectroscopy [29]. As we could not exclude autocatalytic proteolysis of PrP^C, we analyzed the PrP sequence for possible similarities with proteases. We found striking similarities between the structured C-terminal domain of PrP and the catalytic domains of bacterial and eukaryotic signal peptidases. A relation between PrP^C and signal peptidases is further supported by inspection of the three-dimensional structure of PrP(121–231).

2. Results

2.1. Identification of sequence similarities between PrP and signal peptidases

In search for a structural basis for a possible proteolytic activity of PrP^C, a comparison of the mouse PrP sequence with sequences of a variety of proteases did not reveal significant sequence similarities with the major protease families. However, we observed a surprising sequence homology of the C-terminal domain of murine PrP with the catalytic domains of monomeric bacterial signal peptidases, and with the catalytic subunits of eukaryotic signal peptidases. For these membrane-anchored enzymes a three-dimensional structure has so far not been determined. The three previously identified conserved regions in the sequences of bacterial and eukaryotic signal peptidases [30,31] are strikingly homologous to corresponding segments in murine PrP(121–231) (Fig. 1). Within these three consensus regions, PrP is more similar to the catalytic subunits of signal peptidases from mammalians and yeast than to bacterial and mitochondrial signal peptidases (Fig. 1). Specifically, the mouse PrP segment 121–217 is 22.7% identical and 40.9% similar with residues 49–137 of a catalytic subunit of rat signal peptidase (cf. legend of Fig. 1). Seventeen out of the 20 identical residues in mouse PrP and this segment of the rat signal peptidase are invariant in the mammalian PrP sequences [20,21].

The most highly conserved segment among the signal peptidase subunits from rat and yeast, and murine PrP corresponds to the turn between helix 2 and helix 3 in the structure of murine PrP(121–231), i.e. PrP residues 193–197 (cf. Fig. 1). Interestingly, the signal peptidases from *E. coli* and from yeast mitochondria have large insertions of 109 and 28 residues, respectively, before this conserved segment (Fig. 1). Two other strongly conserved regions between PrP(121–220) and the rat signal peptidase which correspond to the PrP segments 121–129 and 159–165, coincide with the antiparallel strands of the β-sheet in the structure of PrP(121–231). A fourth region of high homology between the rat signal peptidase and murine PrP, corresponding to PrP residues 175–186, coincides with the N-terminal half of helix 2 in PrP(121–231). Thus the most highly conserved segments of signal peptidases and PrP are found in the regular secondary structures of PrP(121–231) and the region preceding helix 3. Conversely, non-conserved segments typically correspond to loop regions in the structure of PrP(121–231). For example, the tripeptide insertion of residues 169–171 which is characteristic for PrP when compared to the signal peptidases, is located in the loop between the second β-strand and helix 2, which is the least well defined part in the NMR structure of PrP(121–231) [22]. Variability in this loop is also indicated by the fact that this loop is even longer by 8 amino acids in the chicken prion protein. The only strongly different region between murine PrP and the rat signal peptidase that lies within a regular secondary structure of PrP(121–231) corresponds to the residues 151–157 in the C-terminal half of helix 1. This heptapeptide is absent in the catalytic subunits of the rat and yeast signal peptidases (Fig. 1). Since helix 1 is rather isolated in the structure of PrP(121–231), unusually hydrophilic and does not significantly contribute to the hydrophobic core [22], it seems conceivable that this element of regular secondary structure might be either significantly shorter or possibly absent in an otherwise PrP-like three-dimensional structure of catalytic eukaryotic signal peptidase subunits. The invariant disulfide bridge of mammalian prion proteins between Cys-179 and Cys-214, which forms an important part of the hydrophobic core of PrP(121–231) and connects the helices 2 and 3 [22], is absent in signal peptidases.

2.2. Location of the PrP residues that align with the active-site residues of signal peptidases in the three-dimensional structure of PrP(121–231)

Previous work on the catalytic mechanism of the signal peptidase from *E. coli* indicates that the enzyme represents a new type of serine protease with a catalytic diad consisting of a nucleophilic serine (Ser-90), which is located in the consensus region 1, and a lysine residue (Lys-146) contained in the consensus region 2 [32–34] (Fig. 1). In the eukaryotic signal peptidases this lysine is replaced by histidine [30,31] (Fig. 1). This histidine is also present in PrP (His-177) and is invariant in the mammalian PrP sequences [20,21]. The PrP residue corresponding to the presumed nucleophilic serine of signal peptidases is an invariant tyrosine (Tyr-128) in all mammalian PrP proteins [20,21]. Strikingly, Tyr-128 and His-177 are in close proximity in the NMR structure of PrP(121–231) (Fig. 2). Although we did not observe a direct contact between Tyr-128 and His-177 in the refined, energy-minimized solution

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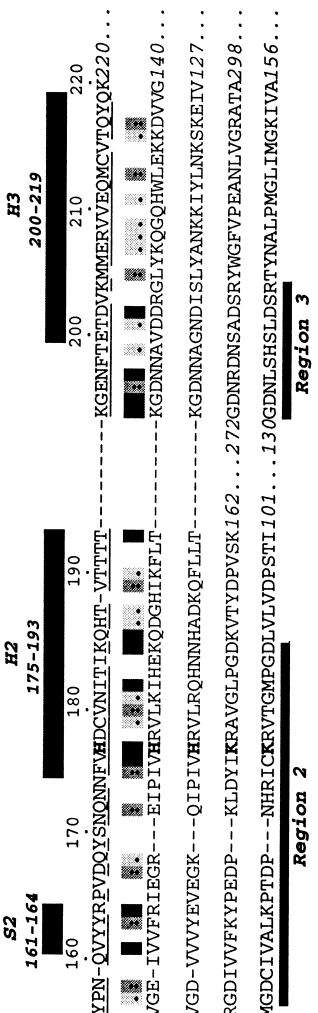
Fig. 1. Sequence comparison of the segment 121–220 of the mouse prion protein (residues 121–220) with the three conserved regions in monomeric bacterial signal peptidases and the catalytic subunits of eukaryotic microsomal signal peptidases. The proteins from *E. coli* (accession numbers: K00426; J03295) yeast mitochondria (Imp-1) (P28627) and catalytic signal peptidase subunits from yeast (Sec-11) (X07694), and rat (L11319) microsomes were chosen as representative enzymes. The positions of the two β-strands and the three α-helices in the NMR structure of murine PrP(121–231) and the corresponding amino acid numbers are indicated at the top of the figure (amino acid numbering according to human PrP [21,22]). The proposed active-site residues of signal peptidases that align with Tyr-128 and His-177 in murine PrP are shown in bold. Invariant residues in mammalian prion proteins are underlined. Dots in the sequences represent additional polypeptide segments and hyphens correspond to lacking amino acids. Identical residues between mouse PrP(121–220) and the rat signal peptidase are emphasized by black vertical bars, strongly similar residues are indicated by colons on dark grey bars, and weak similarities are shown by dots on light grey bars. The previously defined consensus regions 1–3 [30,31] between bacterial and eukaryotic signal peptidases are indicated at the bottom of the figure. The alignment of the murine PrP segment 121–197 with segment 49–137 in the rat signal peptidase was performed with the routine BESTFIT contained in the GCG sequence analysis program (Version 8) [47] with the following default parameters: gap creation penalty: 3.00; gap extension penalty: 0.10. Percentages of identity and similarity were calculated with these parameters.



structure of PrP(121–231), rotation of Tyr-128 about χ^1 and His-177 about χ^1 and χ^2 can readily place the hydroxyl group of Tyr-128 in hydrogen bond distance to the $N^{\delta 1}$ nitrogen of His-177 bond (Fig. 2). The side chains of Tyr-128 and His-177 are thus principally capable to form a charged hydrogen bond. Further inspection of the structure of PrP(121–231) and modelling studies revealed that the side chains of Asn-174 and Asn-173, which are located on the opposite side of the ring of His-177, could form an additional hydrogen bond with the $N^{\varepsilon 2}$ nitrogen of His-177, resulting in a triad with Tyr-128, His-177, and Asn-174 (or Asn-173) (Fig. 2). This modelled local structure is reminiscent of the active sites of cysteine proteases [35]. Asn-174 and Asn-173 in PrP belong to the flexible loop between the second β-strand and helix 2, which contains the above mentioned tripeptide insertion found in all mammalian prion proteins. Asn-173 is invariant in all mammalian prion protein sequences, Asn-174 is either strictly conserved or replaced either by Ser or Thr [20,21].

3. Discussion and outlook

The presently described similarity between mammalian prion proteins and signal peptidases may provide a platform for the design of future experiments to elucidate the cellular function of PrP^C. The 23% sequence identity between the segment 121–217 of murine PrP and residues 49–137 of the rat signal peptidase alone is only slightly below the limit of 25% sequence identity that is generally assumed to be required for a reliable prediction of structural relationships between different proteins. There are however additional factors that support a relationship between PrP^C and these proteases, especially the catalytic subunits of microsomal signal peptidases from eukaryotes. First, the most highly conserved regions of microsomal signal peptidases are also preserved in the prion protein sequence, and so are their order and relative distances. The conserved regions also correspond to regular secondary structures in the tertiary structure of PrP^C. Second, the PrP residues Tyr-128 and His-177, which align with the presumed active-site residues of signal peptidases [32–34] are in close proximity in the three-dimensional structure of PrP(121–231). Third, signal peptidases and PrP^C are both membrane-anchored proteins with similar membrane topology in that they are located at the extracytoplasmic face of the membrane (Fig. 3). The main topological difference is the membrane anchor itself, which is an N-terminal transmembrane domain in the case of signal peptidases and a C-terminal GPI anchor in the case of PrP^C. A C-terminal membrane anchor is however also observed for the signal peptidase of the inner mitochondrial membrane [31] (Fig. 3C). The N-terminal part of the soluble catalytic domain of signal peptidases is best known in the enzyme from *E. coli* where the periplasmic domain starts at residue 76, i.e. 14 residues before the essential Ser-90 [36,37]. This is similar to the C-terminal domain of murine PrP^C [23,26,29], which begins seven residues before Tyr-128.

The serine/lysine diad proposed to be the active site of the *E. coli* signal peptidase is a common motif for the catalytic centers of hydrolases and has also been found in class A β-lactamases [38,39] and LexA-type proteases [40]. In PrP^C the invariant Tyr-128 aligns with the nucleophilic serine of the signal peptidases. Tyrosines are not common nucleophiles in proteases, but are known as essential nucleophiles in type I and type II topoisomerases, where they form a transient phos-

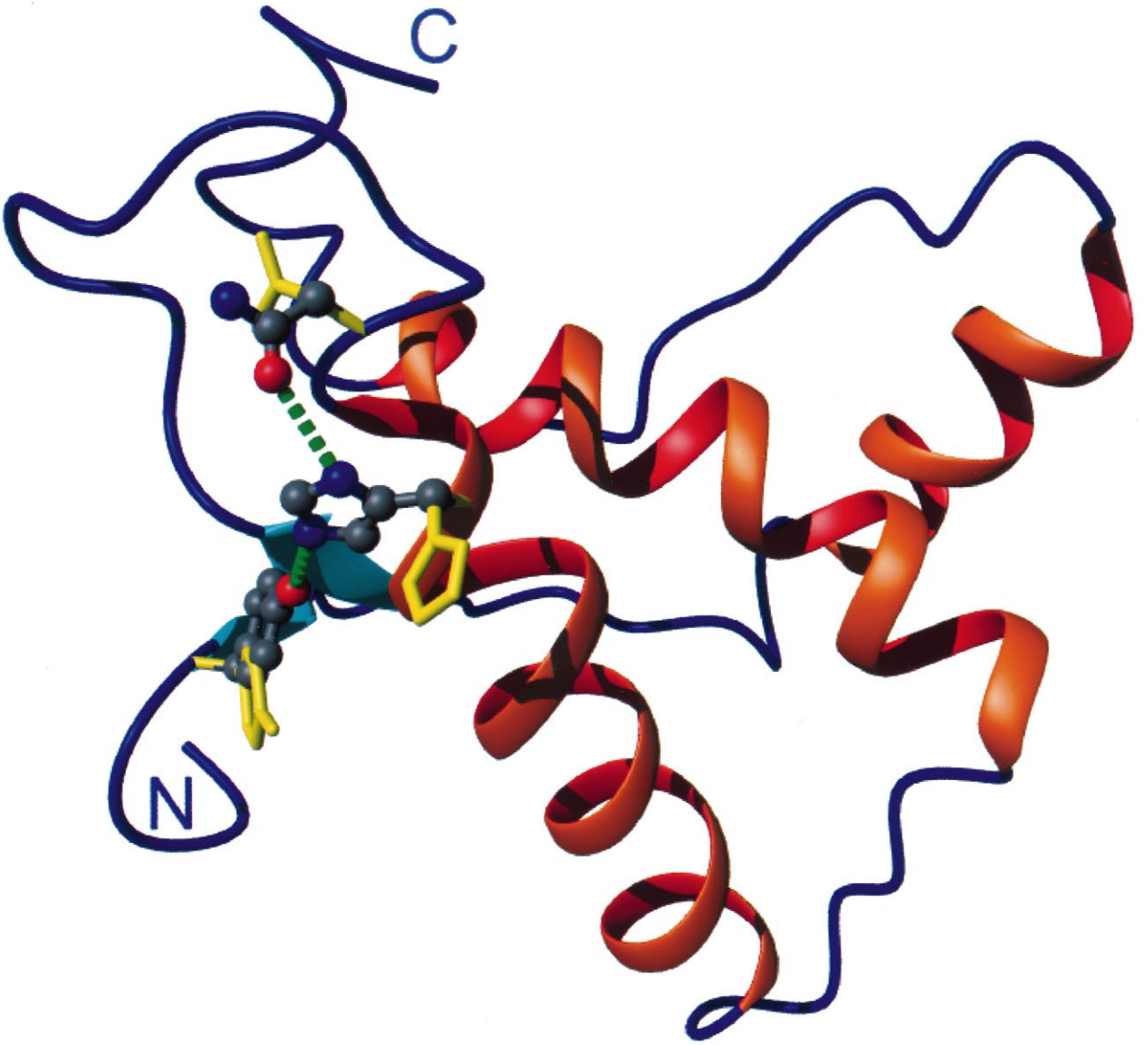


Fig. 2. Location of the PrP residues which align with the active-site residues of signal peptidases in the refined NMR structure of PrP(121–231). A ribbon drawing of the energy-minimized mean solution structure of murine PrP(121–231) [22] is shown with the α-helices in red, the β-strands in light blue and the loop regions in dark blue. The side chains of Tyr-128, His-177 and Asn-154 in the energy-minimized mean structure are shown as yellow stick drawings. A hydrogen bond network reminiscent of the catalytic triad in thiol proteases was generated by rotation of these side chains about the χ^1 angles and χ^2 angles of His-177 and Asn-154. The resulting positions of the side chains are shown as ball and stick models with functional colors, and the hydrogen bonds are shown in green. The figure was generated with the program MOLMOL [48].

photyrosine bond between enzyme and DNA phosphodiester backbone [41–43]. Furthermore, the implicated formation of a triad between Tyr-128, His-177 and Asn-173/Asn-174 in PrP (Fig. 2) is reminiscent of the active sites of cysteine proteases, such as papain with a triad of Cys, His and Asn [35,44].

In a first attempt to demonstrate an enzymatic activity in the C-terminal domain of murine PrP^C, we tested a series of chromogenic protease substrates for trypsin-, chymotrypsin- and elastase-like protease specificities, but could not detect proteolytic activity (S. Hornemann and R. Glockshuber, unpublished data). This leaves the possibilities that PrP^C could have a very narrow specificity for an unknown substrate or is hydrolytically active only in conjunction with other proteins. The latter seems plausible based on observations made with the signal peptidases of Fig. 1: The microsomal signal peptidases from eukaryotes are hetero-oligomers of five different membrane-anchored subunits. Two of these subunits are believed to be the catalytically active proteins. The mitochondrial signal peptidases are heterodimers of two almost identical, catalytically active subunits [31]. PrP^C might similarly associate with a so far unknown protein at the surface of the cellular membrane and might be active only as a heterodimer. An obvious candidate for this presumed partner protein would be the so-called protein X, which has been postulated to be a species-specific protein involved in the conversion of PrP^C to PrP^{Sc} in vivo [45].

Another intriguing aspect of the observed similarities between PrP^C and signal peptidases is the sequence relationship between signal peptidases and the proteases of the LexA family, which also possess a catalytic serine/lysine diad [40,46].

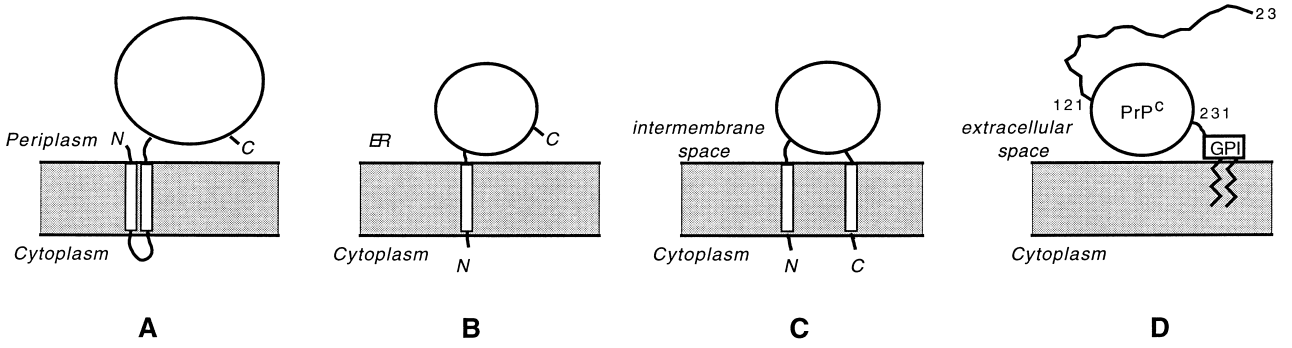


Fig. 3. Schematic drawings of the membrane topologies of selected proteins discussed in this paper. A: Monomeric signal peptidase from *E. coli*. B: Catalytic subunits of microsomal signal peptidases. C: Catalytic subunit (Imp2p) of yeast mitochondria. D: Mammalian cellular prion protein. A–C have been adapted from [31]. The membranes are depicted by grey background and postulated transmembrane helices are shown by white rectangles.

The crystal structure of the UmuD′ protein, a member of this protease family, has been solved [40]. UmuD′ is a homodimer of 137 residues generated by autocatalytic cleavage of UmuD through removal of its N-terminal 24-residue segment (cf. [40]). The X-ray structure of UmuD′ has shown that the subunits of UmuD′ have an unusual β-sheet fold and that the dimers form long filaments in the crystal, which are held together by extended N-terminal tails of the subunits [40]. UmuD′ in the crystal thus shares physical properties with the PrP^{Sc} amyloid [2,9,10,12]. The autocatalytic self-cleavage of UmuD is also reminiscent of our previous finding that the murine PrP segments 95–231 and 107–231 were N-terminally cleaved during expression in the periplasm of *E. coli* [26], so that we cannot a priori exclude the presence of in vivo self-cleaved PrP. Although there is no significant sequence similarity between UmuD′ and the prion protein, a search for possible relationships between the PrP^{Sc} oligomer and the filaments of UmuD′ dimers observed in the X-ray structure of UmuD′ appears to be of potential interest.

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